Pulling the Pin on NPMc+ acute myeloid leukemia

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In this issue of *Haematologica*, Hleihel *et al.*[1] identify the propyl isomerase Pin1 as a key target of retinoic acid (RA, also known as all-*trans* retinoic acid) in NPMc+ acute myeloid leukemia (AML). AML is an aggressive cancer with few effective treatment options and extremely poor outcomes in the majority of cases. Approximately one third of AML patients carry mutations in the *NPM1* gene that encodes the multifunctional protein nucleophosmin. The mutations, collectively termed NPMc+, cluster at the 3' end of the *NPM1* open reading frame and introduce a nuclear export signal that causes relocalization of nucleophosmin from the nucleolus to the cytoplasm.[2] Evidence from clinical trials had suggested that RA treatment may enhance the efficacy of intensive chemotherapy in a subset of NPMc+ patients.[3] Excitingly, two concurrent studies in 2015 implicated RA as a degrader of mutant nucleophosmin,[4,5] but did not elucidate the molecular target of RA responsible for this effect. In their article, Hleihel and colleagues provide novel mechanistic insights using AML cell lines and patients' samples. They further demonstrate synergy between RA and chemotherapy or arsenic trioxide (ATO) in NPMc+ AML, with this synergy being dependent on expression of the protein PML.

RA is a hormone that, at physiological concentrations, regulates a wide array of biological processes by activating gene expression via retinoic acid receptor (RAR) transcription factors. Seminal studies in the 1980s, initially *in vitro* and subsequently in clinical trials, identified the potent efficacy of RA against acute promyelocytic leukemia, a subtype of AML most often characterized by the oncogenic fusion protein PML-RARα. Although the molecular details remain debated, at pharmacological concentrations RA promotes both the transcriptional activation of PML-RARα target genes and the degradation of the fusion protein itself, driving differentiation of promyelocytic blasts to mature neutrophils.[6] Notably, a study by Wei and colleagues in 2014 uncovered that as well as its effects on RAR signaling, RA is also a potent inhibitor of Pin1,[7] a unique enzyme that binds to phosphorylated Ser/Thr-Pro motifs within target proteins and catalyzes their *cis/trans* conformation thereby altering their stability or activity. Known Pin1 targets include RARα, PML and PML-RARα, CyclinD1 and NF-κB.[8]

In the present study, Hleihel *et al.*[1] began by expanding on earlier observations that RA treatment of NPMc+ AML cells leads to NPMc+ proteolysis, P53 activation, differentiation and apoptosis.[4,5] They initially tested whether PML, the essential protein component of PML nuclear bodies, is required for RA activity in NPMc+ AML. PML nuclear bodies are small nuclear matrix-associated structures that provide a molecular docking station for a wide array of interacting proteins. Although seemingly dispensable for life (*Pml* knockout mice are viable), PML nuclear bodies are detectable in most cell types, are regulated by cellular stress and are associated with numerous biological processes and disease states.[9] PML nuclear body formation is dysregulated in NPMc+ AML cells compared with *NPM1* wild-type cells, and the authors found that *PML* knockout in the NPMc+ AML cell line OCI-AML3 abrogates its sensitivity to RA. Analyzing the kinetics of the response to RA by OCI-AML3 cells and NPMc+ primary AML blasts, they unexpectedly found that P53 activation can be untangled from NPMc+ degradation, with the former evident within 2 h of treatment and the latter occurring only after 24-48 h.

These observations in turn prompted the authors to investigate the role of Pin1 in the RA response. Both RA and a structurally distinct Pin1 inhibitor AG17724 triggered stabilization of PML and P53 proteins, solely in NPMc+ cells. These effects could be abrogated by shRNA-mediated Pin1 knockdown, although it is perhaps surprising that Pin1 knockdown itself is tolerated in the *NPM1* mutant context. Importantly, the team found that OCI-AML3 cells and NPMc+ primary AML blasts have increased expression of Pin1 compared with *NPM1* wild-type controls, providing a potential explanation for the selective effects of RA on mutant cells. They went on to validate their findings *in vivo* using an OCI-AML3 xenograft model. As was previously demonstrated *in vitro*,[4,5] RA synergized with both ATO and DNA-damaging chemotherapy, with therapeutic efficacy and NPMc+ degradation dependent on PML expression. Excitingly, two NPMc+ AML patients treated with an RA/ATO combination on a compassionate basis demonstrated a significant albeit incomplete response.

Together, the findings reported in this issue by Hleihel *et al.*, as well as earlier work from their group and others demonstrate the potential for expanding the clinical use of RA beyond acute promyelocytic leukemia. The data support a model whereby RA induces multiple anti-leukemic effects in NPMc+ AML cells, most of which are initiated by and dependent on re-assembly of PML nuclear bodies triggered by inhibition of Pin1. A number of important questions do, however, remain. The mechanism of NPMc+ degradation and the significance of this phenomenon for the therapeutic response beyond OCI-AML3 cells are still unclear. Likewise, the role of RAR signaling in potentiating (or opposing) the Pin1/PML/P53 axis, or indeed AML differentiation in the NPMc+ context, has not been explored. Unbiased methodologies such as pooled CRISPR screening could identify essential nodes of the various aspects of the RA response such as PML stabilization, NPMc+ proteolysis, differentiation and cell death. Further validation using genetically engineered mouse models of NPMc+ AML as well as patient-derived xenografts will also be important for building confidence in the strategy. Collectively, these studies would bring us closer to extending the application of a safe existing drug to an area of unmet need.

Disclosures

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Arsenic and all-*trans* retinoic acid for acute promyelocytic leukemia: yes, it really is as good as it seems

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In this issue of *Haematologica*, Kayser and colleagues report the results of an analysis of outcomes from the National Acute Promyelocytic Leukemia (APL) Observational study (NAPOLEON-Registry; NCT02192619), including 152 non-high-risk APL patients in Germany and France.[1] In their study, which they claim represents a reflection of "real-life" outcomes, these authors specifically focused on APL patients treated upfront with all-*trans* retinoic acid (ATRA) and arsenic, according to the study led by the late Francesco Lo Coco.[2] As with that original protocol, this present study excluded high-risk APL patients.

When Lo-Coco's study was published in 2013, the results seemed almost too good to be true.[2] The event-free survival rate of patients treated with ATRA and arsenic was 97%. In their study of the registry patients, Kayser and colleagues found an almost identical result (event-free survival of 95%, with no patient relapsing after achieving remission. The remarkable efficacy of this regimen seems to be every bit as high even outside of the context of a clinical trial. Two out of 152 patients died during induction, and both were older (64 and 70 years) than typical APL patients. Interestingly, differentiation syndrome was only reported in seven patients (6%), in contrast to the 19% reported in Lo-Coco's study. One wonders whether this is more a reflection of clinicians' comfort in managing and even preventing this condition as they grow more familiar with this regimen over time.

Where to next with APL? Certainly, an oral version of arsenic would expand the use of this combination to many parts of the world lacking access to intravenous medication. It would also represent a major improvement in the quality of life of APL patients, who must trudge through months of daily arsenic infusions. Oral preparations are under investigation,[3] but formulation challenges have thus far been an obstacle to their widespread use.

High-risk APL patients were excluded from these studies, and of course they represent a significant challenge for physicians treating them. In one of the original pilot studies exploring the combination of ATRA and arsenic, gemtuzumab ozogamycin (GO) was used as a cytoreductive agent in the high-risk patients.[4] This highly effective agent is not approved for such use worldwide, but studies to compare its efficacy against anthracyclines are warranted.

Another way to potentially optimize this therapy is to determine how much arsenic is really needed to achieve these high-quality outcomes. The selection of four cycles of consolidation with arsenic was somewhat arbitrary, and no one should lose sight of the fact that arsenic is a group 1 human carcinogen with neurotoxic and hepatotoxic effects.[5] Identifying the minimum necessary number of cycles would be a worthwhile endeavor for the field.

Finally, lest we be too self-congratulatory about how well we are doing with this dreadful malignancy, let us not forget how many patients die of APL before their disease is recognized and treated.[6] At present, in areas of the world that have complete access to standard-of-care leukemia treatment, most APL patients die because their care providers are unknowingly observing the natural history of untreated APL. The failure to recognize APL rapidly is a problem without an immediate solution. However, perhaps in this digital age, there is a ray of hope for this problem. The use of artificial intelligence algorithms combined with digital scanning technology may offer an automated way of identifying an APL patient,[7] leading to the same sort of electronic red flag that occurs when a patient with a low electrolyte or platelet count is evaluated by an emergency room physician. We are probably not far off from that future.

Disclosures

No conflicts of interest to disclose.